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Attorney Docket No.: 4318.224-US

PATENT

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Bisgaard-Frantzen et al. Confirmation No: 7527

Serial No.: 09/902,188 Group Art Unit: 1751

Filed: July 10, 2001 Examiner: To be assigned

For: Amylase Variants

## PENDING CLAIMS

Commissioner for Patents  
Washington, DC 20231

68. A variant of a parent alpha-amylase enzyme, wherein said parent alpha-amylase has an amino acid sequence which has at least 80% homology to SEQ ID NO:3, and wherein said variant comprises deletions at positions equivalent to positions 180 and 181 in SEQ ID NO:3 (using SEQ ID NO:3 for numbering).

69. The variant of claim 68, wherein said parent alpha-amylase has an amino acid sequence which has at least 85% homology to SEQ ID NO:3.

70. The variant of claim 68, wherein said parent alpha-amylase has an amino acid sequence which has at least 90% homology to SEQ ID NO:3.

71. The variant of claim 68, wherein said parent alpha-amylase has an amino acid sequence which has at least 95% homology to SEQ ID NO:3.

72. The variant of claim 68, wherein said variant further comprises amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.

73. An isolated alpha-amylase enzyme comprising an amino acid sequence having an amino acid sequence which has at least 80% homology to SEQ ID NO:3, modified by having deletions at positions equivalent to positions 180 and 181 in SEQ ID NO:3.

74. The alpha-amylase enzyme of claim 73, wherein said alpha-amylase enzyme is further modified by having amino acid substitutions of a cysteine at positions equivalent to 349 and 428 in SEQ ID NO:3.

75. The alpha-amylase of claim 73, wherein said alpha-amylase has an amino acid sequence which has at least 85% homology to SEQ ID NO:3.

76. The alpha-amylase of claim 73, wherein said alpha-amylase has an amino acid sequence which has at least 90% homology to SEQ ID NO:3.

77. The alpha-amylase of claim 73, wherein said alpha-amylase has an amino acid sequence which has at least 95% homology to SEQ ID NO:3.

78. A process for producing an alpha-amylase enzyme, said process comprising:

a) cultivating a host cell having a nucleic acid sequence encoding an alpha-amylase enzyme, said alpha-amylase enzyme comprising an amino acid sequence having at least 80% homology to SEQ ID NO:3 and wherein said alpha-amylase enzyme is modified by having deletions at positions equivalent to positions 180 and 181 in SEQ ID NO:3, wherein said cultivating is performed under conditions conducive to produce the alpha-amylase enzyme, and

b) recovering the alpha-amylase from the culture.

79. The process of claim 78, wherein said alpha-amylase enzyme is further modified by having amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.

80. A DNA construct having a DNA sequence encoding an alpha-amylase enzyme comprising an amino acid sequence of SEQ ID NO:3 and further comprising deletions at positions equivalent to positions 180 and 181 in SEQ ID NO:3.

81. The DNA construct of claim 80, wherein said alpha-amylase enzyme further comprises amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.

82. A recombinant expression vector carrying the DNA construct of claim 80.

83. A cell transformed with the DNA construct of claim 80.
84. A cell transformed with the recombination expression vector of claim 82.
85. A process of producing an alpha-amylase, said process comprising culturing the cell of claim 83 under conditions conducive for the production of the alpha-amylase and recovering the alpha-amylase from the culture.
86. A variant of a parent alpha-amylase enzyme, wherein said parent alpha-amylase has an amino acid sequence which has at least 80% homology to SEQ ID NO:3, and wherein said variant comprises deletions at positions equivalent to positions 179 and 181 in SEQ ID NO:3 (using SEQ ID NO:3 for numbering).
87. The variant of claim 86, wherein said parent alpha-amylase has an amino acid sequence which has at least 85% homology to SEQ ID NO:3.
88. The variant of claim 86, wherein said parent alpha-amylase has an amino acid sequence which has at least 90% homology to SEQ ID NO:3.
89. The variant of claim 86, wherein said parent alpha-amylase has an amino acid sequence which has at least 95% homology to SEQ ID NO:3.
90. The variant of claim 86, wherein said variant further comprises amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.
91. An isolated alpha-amylase enzyme comprising an amino acid sequence having an amino acid sequence which has at least 80% homology to SEQ ID NO:3, modified by having deletions at positions equivalent to positions 179 and 181 in SEQ ID NO:3.
92. The alpha-amylase enzyme of claim 91, wherein said alpha-amylase enzyme is further modified by having amino acid substitutions of a cysteine at positions equivalent to 349 and 428 in SEQ ID NO:3.

93. The alpha-amylase of claim 91, wherein said alpha-amylase has an amino acid sequence which has at least 85% homology to SEQ ID NO:3.

94. The alpha-amylase of claim 91, wherein said alpha-amylase has an amino acid sequence which has at least 90% homology to SEQ ID NO:3.

95. The alpha-amylase of claim 91, wherein said alpha-amylase has an amino acid sequence which has at least 95% homology to SEQ ID NO:3.

96. A process for producing an alpha-amylase enzyme, said process comprising:

a) cultivating a host cell having a nucleic acid sequence encoding an alpha-amylase enzyme, said alpha-amylase enzyme comprising an amino acid sequence having at least 80% homology to SEQ ID NO:3 and wherein said alpha-amylase enzyme is modified by having deletions at positions equivalent to positions 179 and 181 in SEQ ID NO:3, wherein said cultivating is performed under conditions conducive to produce the alpha-amylase enzyme, and

b) recovering the alpha-amylase from the culture.

97. The process of claim 96, wherein said alpha-amylase enzyme is further modified by having amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.

98. A DNA construct having a DNA sequence encoding an alpha-amylase enzyme comprising an amino acid sequence of SEQ ID NO:3 and further comprising deletions at positions equivalent to positions 179 and 181 in SEQ ID NO:3.

99. The DNA construct of claim 98, wherein said alpha-amylase enzyme further comprises amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.

100. A recombinant expression vector carrying the DNA construct of claim 98.

101. A cell transformed with the DNA construct of claim 98.

102. A cell transformed with the recombination expression vector of claim 100.

103. A process of producing an alpha-amylase, said process comprising culturing the cell of claim 101 under conditions conducive for the production of the alpha-amylase and recovering the alpha-amylase from the culture.

104. A variant of a parent alpha-amylase enzyme, wherein said parent alpha-amylase has an amino acid sequence which has at least 80% homology to SEQ ID NO:3, and wherein said variant comprises deletions at positions equivalent to positions 179 and 182 in SEQ ID NO:3 (using SEQ ID NO:3 for numbering).

105. The variant of claim 104, wherein said parent alpha-amylase has an amino acid sequence which has at least 85% homology to SEQ ID NO:3.

106. The variant of claim 104, wherein said parent alpha-amylase has an amino acid sequence which has at least 90% homology to SEQ ID NO:3.

107. The variant of claim 104, wherein said parent alpha-amylase has an amino acid sequence which has at least 95% homology to SEQ ID NO:3.

108. The variant of claim 104, wherein said variant further comprises amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.

109. An isolated alpha-amylase enzyme comprising an amino acid sequence having an amino acid sequence which has at least 80% homology to SEQ ID NO:3, modified by having deletions at positions equivalent to positions 179 and 182 in SEQ ID NO:3.

110. The alpha-amylase enzyme of claim 109, wherein said alpha-amylase enzyme is further modified by having amino acid substitutions of a cysteine at positions equivalent to 349 and 428 in SEQ ID NO:3.

111. The alpha-amylase of claim 109, wherein said alpha-amylase has an amino acid sequence which has at least 85% homology to SEQ ID NO:3.

112. The alpha-amylase of claim 109, wherein said alpha-amylase has an amino acid sequence which has at least 90% homology to SEQ ID NO:3.

113. The alpha-amylase of claim 109, wherein said alpha-amylase has an amino acid sequence which has at least 95% homology to SEQ ID NO:3.

114. A process for producing an alpha-amylase enzyme, said process comprising:

a) cultivating a host cell having a nucleic acid sequence encoding an alpha-amylase enzyme, said alpha-amylase enzyme comprising an amino acid sequence having at least 80% homology to SEQ ID NO:3 and wherein said alpha-amylase enzyme is modified by having deletions at positions equivalent to positions 179 and 182 in SEQ ID NO:3, wherein said cultivating is performed under conditions conducive to produce the alpha-amylase enzyme, and

b) recovering the alpha-amylase from the culture.

115. The process of claim 114, wherein said alpha-amylase enzyme is further modified by having amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.

116. A DNA construct having a DNA sequence encoding an alpha-amylase enzyme comprising an amino acid sequence of SEQ ID NO:3 and further comprising deletions at positions equivalent to positions 179 and 182 in SEQ ID NO:3.

117. The DNA construct of claim 116, wherein said alpha-amylase enzyme further comprises amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.

118. A recombinant expression vector carrying the DNA construct of claim 116.

119. A cell transformed with the DNA construct of claim 116.

120. A cell transformed with the recombination expression vector of claim 118.

121. A process of producing an alpha-amylase, said process comprising culturing the cell of claim 119 under conditions conducive for the production of the alpha-amylase and recovering the alpha-amylase from the culture.

122. A variant of a parent alpha-amylase enzyme, wherein said parent alpha-amylase has an amino acid sequence which has at least 80% homology to SEQ ID NO:3, and wherein said variant comprises deletions at positions equivalent to positions 180 and 182 in SEQ ID NO:3 (using SEQ ID NO:3 for numbering).

123. The variant of claim 122, wherein said parent alpha-amylase has an amino acid sequence which has at least 85% homology to SEQ ID NO:3.

124. The variant of claim 122, wherein said parent alpha-amylase has an amino acid sequence which has at least 90% homology to SEQ ID NO:3.

125. The variant of claim 122, wherein said parent alpha-amylase has an amino acid sequence which has at least 95% homology to SEQ ID NO:3.

126. The variant of claim 122, wherein said variant further comprises amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.

127. An isolated alpha-amylase enzyme comprising an amino acid sequence having an amino acid sequence which has at least 80% homology to SEQ ID NO:3, modified by having deletions at positions equivalent to positions 180 and 182 in SEQ ID NO:3.

128. The alpha-amylase enzyme of claim 127, wherein said alpha-amylase enzyme is further modified by having amino acid substitutions of a cysteine at positions equivalent to 349 and 428 in SEQ ID NO:3.

129. The alpha-amylase of claim 127, wherein said alpha-amylase has an amino acid sequence which has at least 85% homology to SEQ ID NO:3.

130. The alpha-amylase of claim 127, wherein said alpha-amylase has an amino acid sequence which has at least 90% homology to SEQ ID NO:3.

131. The alpha-amylase of claim 127, wherein said alpha-amylase has an amino acid sequence which has at least 95% homology to SEQ ID NO:3.

132. A process for producing an alpha-amylase enzyme, said process comprising:

a) cultivating a host cell having a nucleic acid sequence encoding an alpha-amylase enzyme, said alpha-amylase enzyme comprising an amino acid sequence having at least 80% homology to SEQ ID NO:3 and wherein said alpha-amylase enzyme is modified by having deletions at positions equivalent to positions 180 and 182 in SEQ ID NO:3, wherein said cultivating is performed under conditions conducive to produce the alpha-amylase enzyme, and

b) recovering the alpha-amylase from the culture.

133. The process of claim 132, wherein said alpha-amylase enzyme is further modified by having amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.

134. A DNA construct having a DNA sequence encoding an alpha-amylase enzyme comprising an amino acid sequence of SEQ ID NO:3 and further comprising deletions at positions equivalent to positions 180 and 182 in SEQ ID NO:3.

135. The DNA construct of claim 134, wherein said alpha-amylase enzyme further comprises amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ ID NO:3.

136. A recombinant expression vector carrying the DNA construct of claim 134.

137. A cell transformed with the DNA construct of claim 134.

138. A cell transformed with the recombination expression vector of claim 136.

139. A process of producing an alpha-amylase, said process comprising culturing the cell of claim 137 under conditions conducive for the production of the alpha-amylase and recovering the alpha-amylase from the culture.